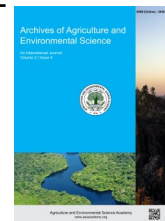




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ORIGINAL RESEARCH ARTICLE



Salinity induced deleterious effects on germination, growth, physiological and biochemical process of two varieties of groundnut (*Arachis hypogaea* L.)

Sharmin Akter¹, Dilruba Yeasmin Jharna¹, Sujan Kanti Mali¹ and Abu Sayeed^{2*} ¹Department of Biochemistry and Food Analysis, Patuakhali Science and Technology University, Patuakhali - 8602, BANGLADESH²Department of Post-Harvest Technology and Marketing, Patuakhali Science and Technology University, Patuakhali - 8602, BANGLADESH*Correspondence author's E-mail: shuvo.nfs.pstu@gmail.com**ARTICLE HISTORY**

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ABSTRACT

The present study was accomplished to find out the effects of salinity on germination, growth, physiological and biochemical processes of two different groundnuts (*Arachis hypogaea* L.), varieties viz., Dacca-1, and Zhinga groundnut. The experiments consisted of five salinity treatments viz., control (no salinity), 3, 6, 9, and 12dS/m during germination and vegetative stage of groundnut varieties. Results showed that germination percentage of both the groundnut varieties was significantly decreased with the increase of salt concentration. During the germination stage, maximum shoot and root length, shoot and root fresh weight and shoot and root dry weight of groundnut varieties were recorded from Zhinga groundnut variety at salt stress. The same result occurred during the vegetative stage of Dacca-1 variety while the Zhinga groundnut variety showed maximum parameters than Dacca-1. Fresh weight, as well as dry weight, of shoots and roots of both the groundnut varieties was also decreased with the increase of salt concentrations while leaf proline concentrations were increased among these varieties. Salinity caused significant ($P<0.001$) reduction in chlorophyll a, chlorophyll b, and total chlorophyll content in both the genotypes. Between two genotypes, Zhinga groundnut recorded higher chlorophyll a, chlorophyll b and total chlorophyll content than Dacca-1. From the studies, it is concluded that the groundnut genotype Zhinga was identified as the tolerant genotype to salt stress than Dacca-1. Overall results indicate that high salinity condition is not suitable for growing the groundnut.

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INTRODUCTION

Salinity is an ever-increasing problem throughout the world and imposes major constraints on food production (Hasegawa *et al.*, 2000; Munns and Tester, 2008). Globally, nearly 100 million hectares of land is affected by salinity which accounts for 6-7% of the total arable land (Munns and James, 2003). Salt-affected soil is defined as one that has been adversely affected in so far as it is no longer suitable for most crops due to the presence of soluble salts (Munns and Tester, 2008). Groundnut is the thirteenth most important food crop of the world; the fourth most important source of edible oil and the third most important

source of vegetable protein (Pal and Pal, 2017; Shalhevet *et al.*, 1969; Sorensen *et al.*, 2004). As a crop of nutritious and economic importance, groundnut seeds contain 40-50% fat, 20-50% protein, and 10-20% carbohydrate depending on the variety (De Pascale and Barbieri, 1997). It is widely used in many countries as cooking oil, digestible protein, minerals, and vitamins, and makes a significant contribution to food safety and poverty alleviation.

In Bangladesh, groundnut is one of the major oilseeds crops with its largest area in the world, but the area and production of this crop are fluctuating mainly due to the climatic variations, and biotic and abiotic stresses (Singh *et al.*, 2004). Salinity can limit

the growth and development of plants (Satu and Ahmad, 2019; Shannon, 1996). Among many reasons ascribed for the lower productivity of groundnut, salinity is an important abiotic stress which significantly affects seedling, vegetative, and reproductive growth, seed quality, and yield. The climatic and edaphic conditions of coastal areas of Bangladesh are suitable for growing groundnut, but this is limited by high soil salinity and high pH (Satu and Ahmad, 2019). Bangladesh is thought to be one of the most vulnerable countries of the world to climate change and sea-level rise (Sayeed et al., 2020). There are several numbers of environmental issues and problems that are hindering the development of Bangladesh. Salinity is such an environmental issue that is expected to be exacerbated in the future by climate change and the rise of sea levels. Salinity creates an unfavorable environment and hydrological condition that constrains regular crop production throughout the year. Salinity is a growing issue in many parts of the world where irrigation is required to sustain efficient agricultural production (Yufeng, 2006). Many factors such as low rainfall and higher surface evaporation, salty water irrigation, contamination of water, and improper agricultural practices are mainly responsible for salinity (Foolad, 2004). The salinity of arable land is an increasing problem under irrigated where rainfall is insufficient to leach down the salts from the root zone and it has been recognized as a significant factor in declining crop productivity (Pandya and Subbaiah, 2017). The main objective of the present study was to identify which variety is salt tolerant by examining the effects of salinity on seed germination rate and growth parameters (shoot height, root length, fresh and dry biomass of shoot and root) under different known concentrations of salt (NaCl) solution.

MATERIALS AND METHODS

Seeds of two different varieties of groundnut (*Arachis hypogaea* L.), Zhinga and Dacca-1, were collected from Bangladesh Agricultural Research Institute (BARI), Joydevpur, Gazipur. Before germination, seeds were surface-sterilized with alcohol. Then, the seeds were left for three days for germination in a Petri-dish with autoclaved distilled water. Germination test was maintained five salinity levels such as 0, 3, 6, 9, and 12 dS m⁻¹. Germination test was carried out in petri-dishes of 15 cm in diameter. Two layers of filter paper were set at the bottom of the petri-dishes. Twenty seeds were placed on filter paper bed and 10ml of treatment solutions of different salinity levels were poured in each petri dish to immerse the seeds partially for ensuring proper aeration. The petri-dishes were placed on a table in the laboratory. The seeds were allowed to germinate at room temperature (25±2°C). Distilled water was added to each Petri dish every day as required. The germination percentage of seeds was recorded after 3 days because before that no seeds were germinated. The design of the study involved both laboratory and net house experiments. The laboratory experiment was made to screen best performing genotypes to assess the salt tolerance of groundnut at germination and seedling growth. The net house experiment was conducted from January to June,

2019 using plastic pot in a net house at Patuakhali Science and Technology University, with three replications and five salt treatments (0, 3, 6, 9, and 12 dS m⁻¹). Here, sandy loam soil was collected from the research farm of Patuakhali Science and Technology University and sifted through a four mm sieve to get rid of large particles. The seeds were surface sterilized in the same way as the laboratory experiment. The tap water was measured by the EC meter before the experiment. The pots were filled with 5kg sandy loam soil and five sterilized seeds were sown in each pot, and irrigated with tap water for three weeks at three days interval after sowing the groundnut crops. The seedlings were thinned to five plants per pot at 15 days after emergence. Then, each pot was treated with 500 ml of selected salt solutions for one month in three days intervals. An equal amount of tap water was used to irrigate the control pots for the same duration. Shoots and roots were separated from the plants. Shoot and root length, fresh weight and dry weight of shoot and root were recorded. Dry weight was measured after drying in an oven at 60 °C for 24 h.

Proline content

Proline content was determined using the method of Bates et al. (1973). 1g fresh leaf was used for the extraction of proline; optical density was recorded at 520nm wavelength by spectrophotometer.

Chlorophyll content

Chlorophyll content was determined using the methods of (Arnon, 1949). It involved 0.5 g of fresh leaf tissue from the fourth youngest fully expanded leaf extracted in 10 mL of 80% acetone for seven days in the dark. The absorbance of the extract was measured using a spectrophotometer (Model Novaspec II, Pharmacia Biotech, Cambridge, England) at 645 and 663 nm to determine the content of chlorophylls a and b respectively. Total chlorophyll was calculated by adding chlorophylls a and b.

RESULTS AND DISCUSSION

Salinity effects on seed germination

The salt treatments significantly ($P<0.01$) reduced germination as compared to the Control in both genotypes. Although both genotypes did not attain 100% germination in the control, the effect of NaCl salinity was evident with an increase in the electrical conductivity. The effects of different concentrations of NaCl solution on germination of groundnut seeds are shown in Table 1. An increase in NaCl concentration reduced germination percentage and significantly decreased percent seed germination. The results indicate that both the groundnut genotypes can tolerate up to 6 dS m⁻¹ salinity during seed germination. It is noticeable that the Zhinga genotype was less affected by higher NaCl salinity (9 and 12 dS m⁻¹) than the Dacca-1, indicating that seeds of Zhinga genotypes were more osmotolerant compared to Dacca-1 (Table 1).

Table 1. Germination percentage of groundnut genotypes as affected by different levels of salt stress.

Salt conc. (dS m ⁻¹)	Total Seed		3 rd Day (%)		4 th Day (%)		5 th Day (%)	
0	20	20	65	60	100	95	100	95
3	20	20	55	50	85	75	85	80
6	20	20	35	30	60	40	70	55
9	20	20	15	15	35	30	60	45
12	20	20	0	0	10	5	10	10

Table 2. Effects of salt solutions on the root length of groundnut.

Salt conc. (dS m ⁻¹)	Root length (cm)	
	Zinga	Dacca-1
0	7.4±.59	5.45±.43
3	4.7±.37	3.95±.31
6	3.33±.26	3.23±.25
9	2.33±.18	2.23±.17
12	1.17±.09	1.16±.09
F-ratio	241.15	171.567
P-value	<.001	<0.001

Root length

Salinity had a significant ($P<0.01$) effect on root lengths in both genotypes. The effect of different salinity levels on root length of groundnut ranged between (1.16±.09) cm in 12 dS m⁻¹ and (7.4±.59) cm in the control treatment. The highest root length was obtained from 0 dS m⁻¹ (7.4±.59) cm at Zinga groundnut and the lowest root length was obtained from 12 dS m⁻¹ (1.16±.09) cm at Dacca-1 groundnut. Generally, increasing salinity levels to 3, 6, 9, and 12 dS m⁻¹. NaCl significantly reduced RL when compared with the control treatment. Root length decreased with increasing salinity levels, as reported by Aydinsakir et al. (2015). Root length is one of the most important parameters for salinity because roots are in direct contact with soil and absorb water from the soil and the shoots supply it to the aerial parts of the plant (Jamil and Rha, 2004) (Table 2).

Shoot fresh and dry weight

The effects of different salinity levels on plant fresh and dry weight are displayed in Table 3. It showed significant difference ($P = 0.004$ and $P = 0.001$, respectively). The maximum plant fresh weight was noted in control (1.67±.13g) at Zinga groundnut and the lowest fresh weight was in 12 dS m⁻¹ (0.8±.06g) at Dacca-1 groundnut. The highest plant dry weight was noted in control (0.51±.04g) at Zinga groundnut, and the lowest plant fresh weight in 12 dS m⁻¹ (0.05±.003g) at Dacca-1 groundnut. It was observed that fresh and dry weights decreased gradually from control to 12 dS m⁻¹ salt treatment. Reduction in plant shoot and root fresh weight in response to salt stress has been reported for other crops, such as soybean (Zaidi and Sing, 1993), chickpea (Khalid et al., 2001), cowpea (Düzdemir et al., 2009), broad bean (De Pascale and Barbieri, 1997), black cumin (Hajar et al., 1996), melon (Sivritepe et al., 2005) and tomato (Yurtseven et al., 2005).

Root fresh and dry weight

The effects of different salinity levels on root fresh and dry weight are presented in Table 4. Root fresh and dry weight showed a significant difference ($P = 0.003$ and <0.001). Root fresh weight ranged from 0.09±.007g to 0.033±.002g and dry

weight from 0.019±.001g to 0.003±.0001g. The highest root fresh weight was obtained in control (0.09±.007g) at Zinga groundnut and the lowest root fresh weight in 12 dS m⁻¹ (0.033±.002g) at Dacca-1 groundnut. A similar trend was found in the case of root fresh and dry weights. High salinity may inhibit root and plant elongation due to slowing down the water uptake by the plant which might be another reason for this decrease (Werner and Finkelstein, 1995). Salinity can rapidly inhibit root growth and hence the capacity of water uptake and essential mineral nutrition from soil (Neumann, 1995).

Proline content

The effects of salinity on the proline contents of groundnut plants are shown in Table 4. In the leaf tissues, the proline contents differed significantly ($P<0.001$) among different concentrations of salt. Proline accumulation in root tissue increased with the increase of salt concentrations (Satu and Ahmad, 2019). The lowest proline content was (0.399±0.03 µg/g) recorded in control at Dacca-1 and the highest proline content was (2.375±0.018µg/g) obtained in 12 dSm⁻¹ salt treatment at Zinga groundnut. Cha-Um and Kirdmanee (Cha-Um and Kirdmanee, 2009) have reported higher proline concentration in lentil plants under salt stress conditions (Figure 1).

Chlorophyll content

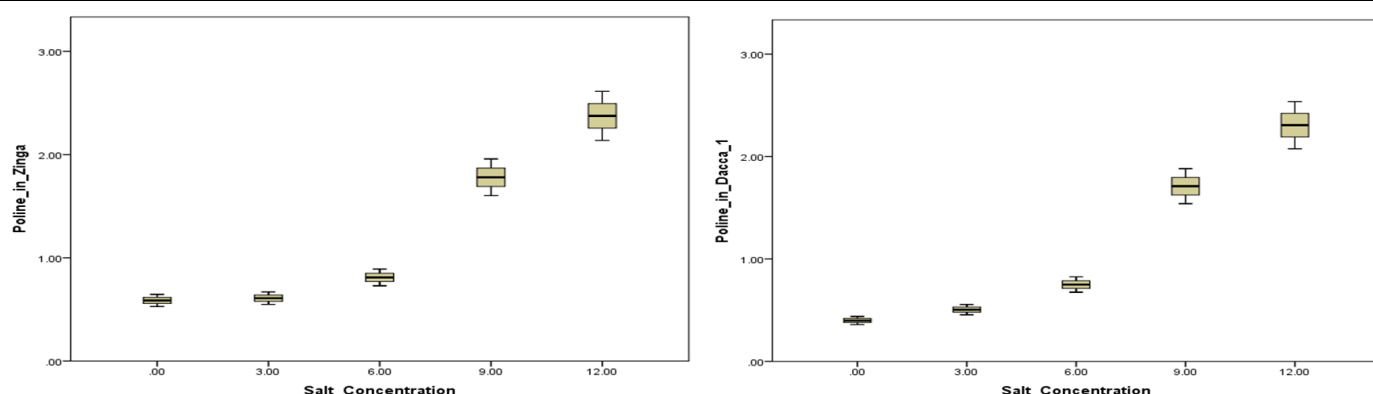
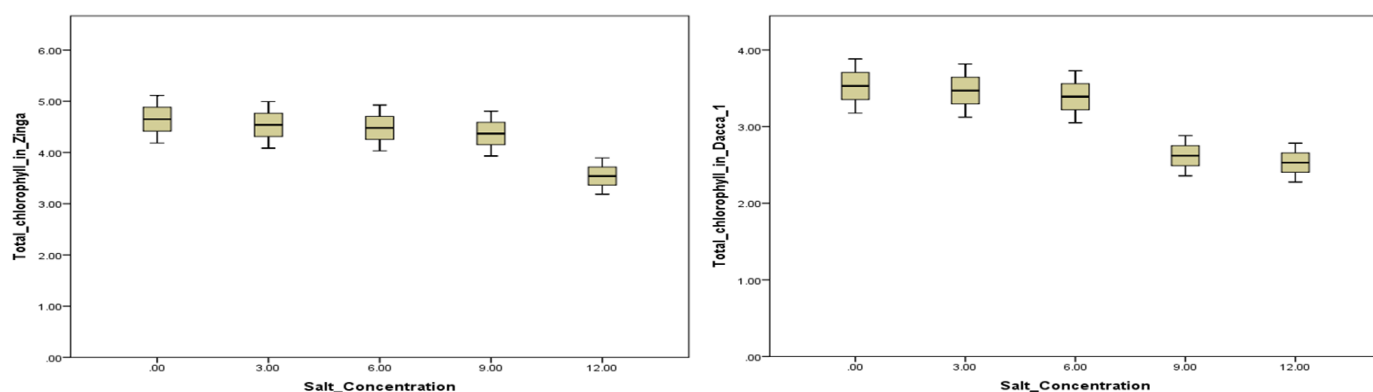
Salinity caused significant ($P<0.001$) reduction in chlorophyll a and b, and total chlorophyll content in both groundnut genotypes, respectively (Table 4). Between two genotypes, Zinga groundnut recorded higher chlorophyll a, chlorophyll b and total chlorophyll content than Dacca-1. At the end of the experiment, the Dacca-1 control plants were generally significantly ($P<0.001$) different in chlorophyll a, chlorophyll b and total chlorophyll content from the 6 dS m⁻¹ plants, while in Zinga groundnut the differences were not significant. Reduction in chlorophyll concentrations is probably due to the inhibitory effect of the accumulated ions of various salts on the biosynthesis of the different chlorophyll fractions (Neumann et al., 2010) (Figure 2).

Table 3. The effect of NaCl salinity on root length, root dry weight, shoot dry weight, and leaf area in Kakamega and Mumias bambara groundnut landraces.

Salt conc. (dS m ⁻¹)	Shoot fresh weight (g/plant)		Shoot dry weight (g/plant)		Root fresh weight (g/plant)		Root dry weight (g/plant)	
	Zinga	Dacca-1	Zinga	Dacca-1	Zinga	Dacca-1	Zinga	Dacca-1
0	1.67±.13	1.36±.1	0.51±.04	0.15±.01	0.09±.007	0.065±.005	0.019±.001	0.015±.0005
3	1.07±.08	1.06±.08	0.15±.01	0.12±.009	0.06±.004	0.059±.004	0.017±.001	0.015±.0005
6	1.02±.08	1.01±.07	0.1±.007	0.09±.007	0.053±.004	0.042±.003	0.015±.001	0.011±.0003
9	1±.07	0.9±.07	0.09±.007	0.08±.006	0.048±.003	0.037±.002	0.011±.008	0.007±.0002
12	0.9±.07	0.8±.06	0.07±.005	0.05±.003	0.039±.003	0.033±.002	0.005±.004	0.003±.0001
F-ratio	55.58	33.05	446.07	109.09	82.57	66.13	120.66	172.97
P-value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Table 4. The effect of salinity on Proline content, chlorophyll a, b and total content in leaves of Zinga and Dacca-1 groundnut genotypes.

Salt conc. (dS m ⁻¹)	Proline content		Chlorophyll a		Chlorophyll b		Total chlorophyll	
	Zinga	Dacca-1	Zinga	Dacca-1	Zinga	Dacca-1	Zinga	Dacca-1
0	0.587±.04	0.399±.03	2.62±.2	1.45±.11	1.99±.15	1.73±.13	4.65±.36	3.53±.27
3	0.609±.04	0.505±.03	2.53±.2	1.42±.11	1.95±.15	1.7±.13	4.54±.35	3.47±.27
6	0.81±.06	0.75±.05	2.23±.17	1.17±.09	1.92±.15	1.5±.11	4.48±.35	3.39±.26
9	1.78±.14	1.71±.13	2.19±.17	1.17±.09	1.85±.14	1.25±.09	4.37±.34	2.62±.2
12	2.375±.18	2.306±.18	1.85±.14	1.09±.08	1.19±.09	1.01±.09	3.54±.27	2.53±.2
F-ratio	254.307	302.51	14.11	13.276	27.35	35.115	8.45	19.5
P-value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

**Figure 1.** Proline content of groundnut (Zinga and Dacca- 1) as influenced by salinity at vegetative stage.**Figure 2.** Total Chlorophyll content of groundnut (Zinga and Dacca- 1) as influenced by salinity at vegetative stage.

Groundnut is moderately sensitive to salinity stress can tolerate 3 dS m⁻¹ (Shalhevet et al., 1969). The salt treatments significantly reduced germination as compared to the control in groundnut genotypes. Increases in NaCl concentration progressively inhibited seed germination and seedling growth as like the other studies (Pal and Pal, 2017; Satu and Ahmad, 2019). Salinity is one of the most significant environmental challenges limiting plant

productivity, particularly in arid and semi-arid climates (Zaidi and Sing, 1993). Soil salinity affects about 800 million hectares of arable lands worldwide (Munns and Tester, 2008). A soil is considered to be saline when the electric conductivity (EC) of the soil solution reaches 4 dS m⁻¹ (equivalent to 40 mM NaCl), generating an osmotic pressure of about 0.2 MPa and significantly reducing the yields of most crops (Munns and Tester, 2008).

Between two genotypes Zhinga groundnut showed the highest germination percentage in all salinity levels. These results are similar to those reported by (Jamil and Rha, 2004; Pandya and Subbaiah, 2017). The effect of salinity level up to 3 dS/m was negligible and there was no difference between treated and control groups. Above this, all the indices were considerably influenced by salinity. Salt levels of 9 dS m⁻¹ and 12 dS m⁻¹ had some negative significant ($P < 0.001$) effect on growth indices. The salt treatments reduced germination percentage, shoot and root length, shoot and root weight as compared to the control in groundnut genotypes. The salt treatments reduced the length of the shoot and shoot fresh weight in all the varieties of groundnut. Hernhdeza and Taufiq reported that NaCl had different effects on shoot fresh and dry weight of groundnut (Hernhdeza *et al.*, 1995; Taufiq *et al.*, 2017). The most common salinity effect is a general stunting of plant growth. Naseer (Naser *et al.*, 2009) reported that the reduction in SL may be due to excessive accumulation of salt in the cell wall, which modifies the metabolic activities and limits the cell wall elasticity. Further, the secondary cell wall appears sooner and the cell wall becomes rigid, as a consequence of which the turgor pressure efficiency in cell enlargement decreases. This process may cause the SSL to remain small (Huang and Redmann, 1995). The possible reason for the reduced shoot development could also be due to the toxic effects of the NaCl used as well as the unbalanced nutrient uptake by the seedlings. It seems that the reduction in SFW may be due to decreasing water uptake by seedling under salinity.

The plant height and root length are the most important parameters for salinity because roots are in direct contact with soil and absorb water from the soil and shoot supply it to the rest of the plant (Jamil and Rha, 2004; Satu and Ahmad, 2019). For this reason, root length and plant height provide an important clue to the response of plants to salt stress (Jamil and Rha, 2004). Root length and plant height decreased with increasing salinity levels; at 4 dS m⁻¹ they decreased drastically. The reason for reduced plant and root development may be due to toxic effects of the salt sources used as well as unbalanced nutrient uptake by the seedlings. High salinity may inhibit root and plant elongation due to slowing down the water uptake by the plant may be another reason for this decrease (Werner and Finkelstein, 1995). Neumann (Neumann, 1995) indicated that salinity can rapidly inhibit root growth and hence the capacity of water uptake and essential mineral nutrition from the soil. These results are similar to those reported by Gupta and Srivastava, Huang, Foolad, Jamil, and Aydinsakir (Aydinsakir *et al.*, 2013; Foolad, 2004; Gupta and Srivastava, 1989; Huang and Redmann, 1995; Jamil and Rha, 2004). It was found that during the germination stage, the maximum shoot length, root length, shoot fresh weight; root fresh weight, shoot dry weight and root dry weight were recorded from Zhinga groundnut at salt stress. Salinity caused significant ($P < 0.001$) reduction in chlorophyll A, chlorophyll B, and total chlorophyll content in both genotypes. Between two genotypes, Zhinga groundnut recorded higher chlorophyll a, chlorophyll b and total chlorophyll content than Dacca-1. The dramatic increase in proline content in the leaves

under salinity stress was consistent with the role of proline as a compatible solute for osmotic adjustment during osmotic shock. The study showed that the proline content of genotypes was increased in salinity stress. Overall, results indicate that the growth parameters of both groundnut genotypes plants were markedly affected by 12 dS m⁻¹ salt treatments indicating that high salinity is not suitable for the cultivation of groundnut.

Salinity is one of the most serious crop productivity limiting factors, with negative effects on germination, plant vigor and crop yield. The detrimental effects of high salinity on plants can be observed at the whole-plant level in terms of plant death and/or decrease in productivity. The findings of this study show that there were variations in the performance of groundnut genotypes at the different concentration levels of NaCl used during the study. The salt treatments significantly reduced germination as compared to the control in both groundnut genotypes. Generally, in the control condition, the percentage of germination was found the highest and it gradually decreased with the increase of salt concentration. The deleterious salt influences on the plant can be along with their physiological and biochemical processes in plants. The chlorophyll content and fluorescence of chlorophyll usually decrease in leaves under salt stress. Chlorophyll fluorescence can monitor the function of the photosynthetic machinery to salt stress. Zhinga groundnut recorded higher chlorophylls a and b and total chlorophyll content than Dacca-1 at the highest salinity level. Reducing sugar, non-reducing sugar and total sugar content significantly increased due to salinity. The dramatic increase in proline content in the leaves under salinity stress was consistent with the role of proline as a compatible solute for osmotic adjustment during osmotic shock. The study showed that the proline content of both genotypes is increased in salinity stress. From the studies, it is concluded that the groundnut variety Zhinga was identified as the most tolerant variety to salt stress and Dacca-1, the most sensitive one.

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Conclusion

Salinity is one of the most serious crop productivity limiting factors, with negative effects on germination, plant vigor and crop yield. The detrimental effects of high salinity on plants can be observed at the whole-plant level in terms of plant death and/or decrease in productivity. The findings of this study show that there were variations in the performance of groundnut genotypes at the different concentration levels of NaCl used during the study. The salt treatments significantly reduced germination as compared to the control in both groundnut genotypes.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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